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13. ABSTRACT ***(Maximum 200 Words)***

This entire project is based on the hypothesis that we can design and develop new synthetic triterpenoids that would eventually be useful for chemoprevention of prostate cancer. With the known importance of oxidative stress and the known involvement of the enzymes, inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), in the process of carcinogenesis in several other organs, and our own preliminary findings that new synthetic triterpenoids can block de novo induction and synthesis of both these enzymes, there is now a sound mechanistic basis for this hypothesis. Furthermore, since we have already shown that new synthetic triterpenoids can inhibit cell growth, without evident cytotoxicity, in non-malignant prostate epithelial cells, we believe that it will be possible to design and synthesize even more effective triterpenoids for this purpose. Finally, it is critical that a receptor (or receptors) for triterpenoids be defined, since these are presently unknown.

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Michael B. Spoon

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INTRODUCTION

There is a major need for new drug discovery in the field of prostate cancer. There is a particular need for developing new agents to prevent this disease, since screening techniques are now identifying large numbers of men with early, pre-malignant lesions in their prostate. Such lesions do not require surgery and are not treatable with conventional chemotherapy. However, men with this type of pre-malignant condition are at definite risk for future development of invasive, metastatic prostate cancer which is life-threatening. This project will attempt to develop a new class of molecules, the triterpenoids, as chemopreventive agents which could eventually be used to prevent prostate cancer in men at high risk.

BODY

TASK 1 To synthesize new triterpenoids and test them as inhibitors of de novo synthesis of iNOS and Cox-2

a) continue efforts to make new triterpenoid molecules
b) perform assays by Northern blot analysis to determine effects on transcription of iNOS and COX-2 genes
c) perform assays by Western blot analysis to determine effects on synthesis of new iNOS and COX-2 proteins

TASK 2 To evaluate new triterpenoids as inhibitors of growth of prostate cells.

a) perform assays on NRP-152 and NRP-154 prostate cells

Synthesis of New Triterpenoids

We have made excellent progress in the synthesis of new synthetic triterpenoids during the past year. We are attaching a preprint, entitled "Novel Synthetic Oleanane Triterpenoids: A Series of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages" by Tadashi Honda, BarbieAnn V. Rounds, Lothar Bore, Frank G. Favaloro, Jr., Gordon W. Gribble, Nanjoo Suh, Yongping Wang, and Michael B. Sporn, which describes the synthesis of more than 20 new triterpenoids, and the ability of these triterpenoids to inhibit de novo synthesis of iNOS. This preprint will be published shortly in *Bioorganic & Medicinal Chemistry Letters*, and support from this grant, DAMD17-98-1-8604, is specifically acknowledged.

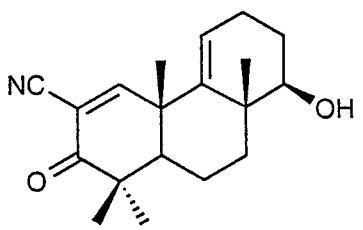
In addition to the pentacyclic triterpenoids described in the above preprint, we have also been attempting to simplify the structural requirements for inhibition of de novo synthesis of iNOS. Accordingly, we have just completed the synthesis of 5 new tricyclic structures, which are triterpenoid-like bis-enones. The structures of these new molecules, labeled TBE-001A, TBE-002A, TBE-003A, TBE-004A, and TBE-005A are attached. Please note that all 5 of these new molecules are simplified structural analogues of the highly potent triterpenoid, CDDO, which is described, in the above-mentioned preprint.

Biological Assays of New Triterpenoids

1) Northern blots to measure suppression of de novo synthesis of iNOS mRNA. Data on page 13 show that when NRP-152 or NPR-154 prostate cells are treated with the combination of LPS (30 ng/ml) and TPA (30 ng/ml) there is major induction of new mRNA for iNOS. Simultaneous treatment of these prostate cells for 10 hours with CDDO (1 micromolar) or the triterpenoid, TP-82 (3 micromolar) causes almost total inhibition of this induction of iNOS. Page 13 also shows that the parent substance for the synthesis of CDDO or TP-82, namely oleanolic acid, is inactive in this regard.

2) Western blots to measure suppression of de novo synthesis of iNOS protein. Data on pages 14 - 18 show results with Western blots for iNOS protein, which confirm the Northern blot data shown in the previous section. Moreover, page 14 also shows that CDDO and TP-82 are essentially inactive in blocking de novo induction of COX-2 protein in NRP-152 cells.

3) Biological activity of new tricyclic bis-enone compounds (TBEs). Data on pages 19 and 20 show our first measurements of the biological activity of the new TBEs described above (TBE-1A, TBE-2A, TBE-3A, TBE-4A, and TBE-5A). The first measurements that we performed were to measure inhibition of nitric oxide production in primary macrophages that had been treated with interferon-γ (page 19). Peritoneal macrophages were treated with interferon–γ (40 ng/ml) to induce nitric oxide production simultaneously these macrophages were incubated with either CDDO or one of the 5 TBEs for 48 hours and then nitric oxide in the supernatant was measured by the Griess Reaction. Page 19 shows that TBE-5A has substantial inhibitory activity (greater than 50% at 100 nanomolar), while the other TBEs are somewhat less potent, although all show highly significant activity at 1 micromolar. TBEs have also been found, for the first time, to have substantial anti-proliferative activity on prostate cells. Thus, on page 20 we show that TBEs 3A, 4A, and 5A all cause almost total inhibition of thymidine incorporation into DNA in NRP-152 cells at a concentration of 1 micromolar.

TBE-Number		TBE-001A	
Structure		Date	9 /3/ 99
		Quantity	10.0 mg
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		Solubility	
		H_2O EtOH DMSO >10 mg/mL ☐ ☐ ☐ 1 mg/mL ☐ ☑ ☑ 0.1 mg/mL ☐ ☐ ☐ <0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{19}H_{25}NO_2$		
Molecular Weight	299.41	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	> 95 % (by NMR)	Note No. Page	FF-I-208-1
IUPAC Name	(±)-3-Cyano-8β-hydroxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthren-2-one		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

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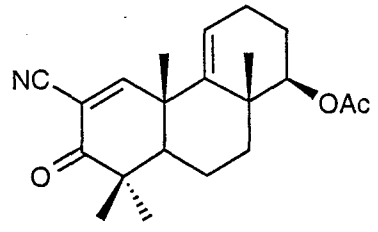		Quantity	10.0 mg
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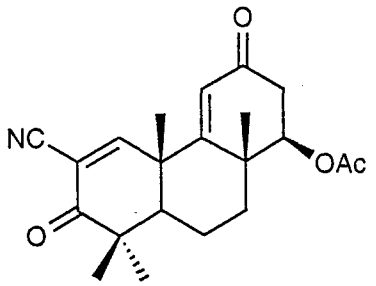
		Solubility	
		H_2O / EtOH / DMSO >10 mg/mL ☐ ☐ ☐ 1 mg/mL ☐ ☑ ☑ 0.1 mg/mL ☐ ☐ ☐ <0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{21}H_{27}NO_3$		
Molecular Weight	341.45	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	> 95 % (by NMR)	Note No. Page	FF-I-191-2
IUPAC Name	(±)-3-Cyano-8β-acetoxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthren-2-one		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number			TBE-003A
Structure		Date	9/9/99
		Quantity	7.6 mg
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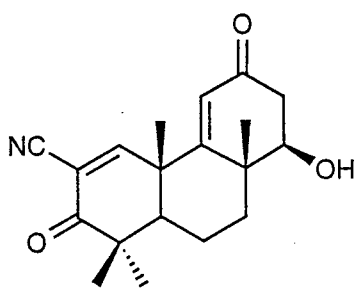
Formula	$C_{21}H_{25}NO_4$	Spectra	
Molecular Weight	355.43		
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	> 95 % (by NMR)	Note No. Page	FF-I-206-2
IUPAC Name	(±)-3-Cyano-8β-acetoxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthrene-2,6-dione		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number		TBE-004A	
Structure		Date	9/13/99
		Quantity	7.6 mg
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		Solubility	
		H_2O / EtOH / DMSO	
		>10 mg/mL ☐ ☐ ☐	
		1 mg/mL ☐ ☑ ☑	
		0.1 mg/mL ☐ ☐ ☐	
		<0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{19}H_{23}NO_3$		
Molecular Weight	313.40	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	95 % (by NMR)	Note No. Page	FF-I-207L
IUPAC Name	(±)-3-Cyano-8β-hydroxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthrene-2,6-dione		
Remarks	Unstable against air. Keep it under argon. Store in freezer.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number		TBE-005A	
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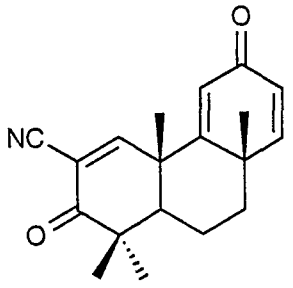		Quantity	7.5 mg
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		Solubility	
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Formula	$C_{19}H_{21}NO_2$		
Molecular Weight	295.38	Spectra	
mp		NMR☑ MS☐ IR☐ EA☐	
$[\alpha]_D$			
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	95 % (by NMR)	Note No. Page	FF-I-207H
IUPAC Name	(±)-3-Cyano-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,8a,9,10,10aα-octahydrophenanthrene-2,6-dione		
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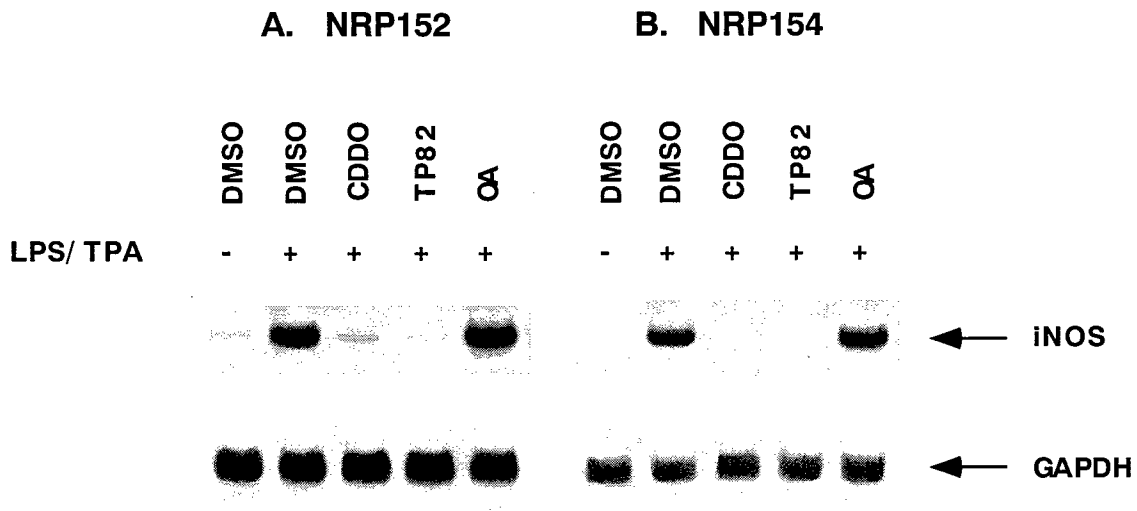
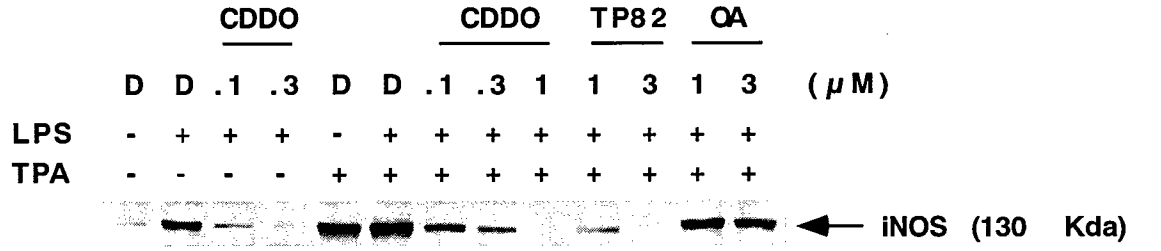
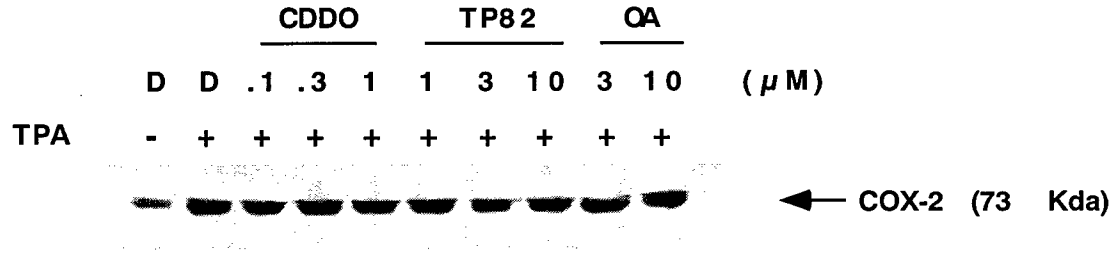
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG



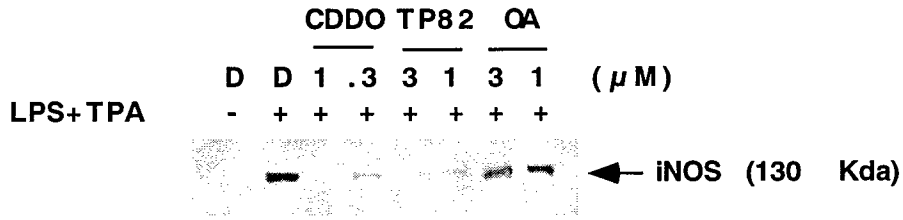
NRP152 cells were treated with LPS (30 ng/ml) and TPA (30 ng/ml) in the presence or absence of different concentrations of compounds (CDDO, 1 μM; TP-82, 3 μM; oleanolic acid, OA, 3 μM) for 10 h. mRNA were obtained and used for Northern analysis for iNOS expression.

A. NRP152



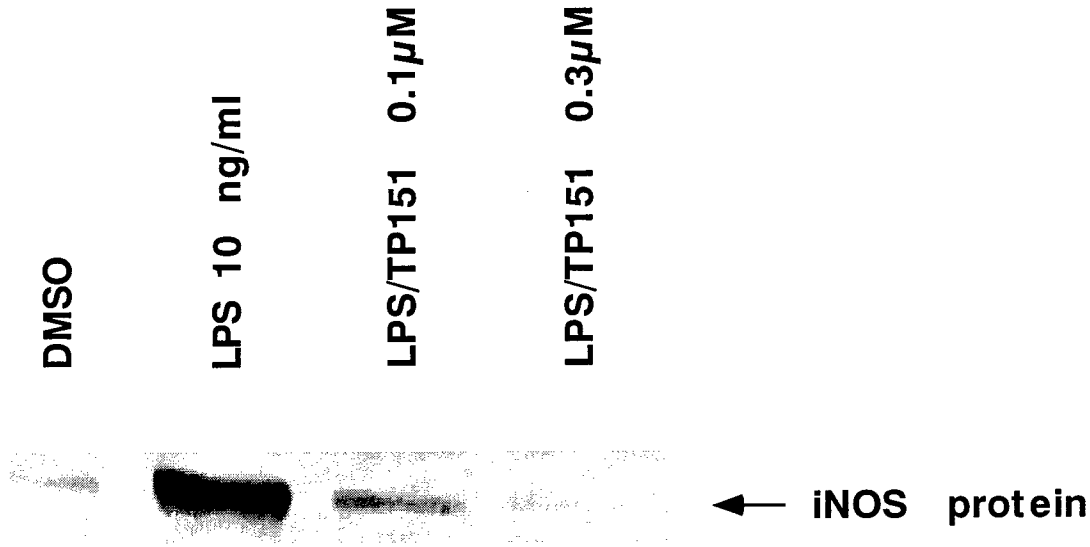


B. NRP154



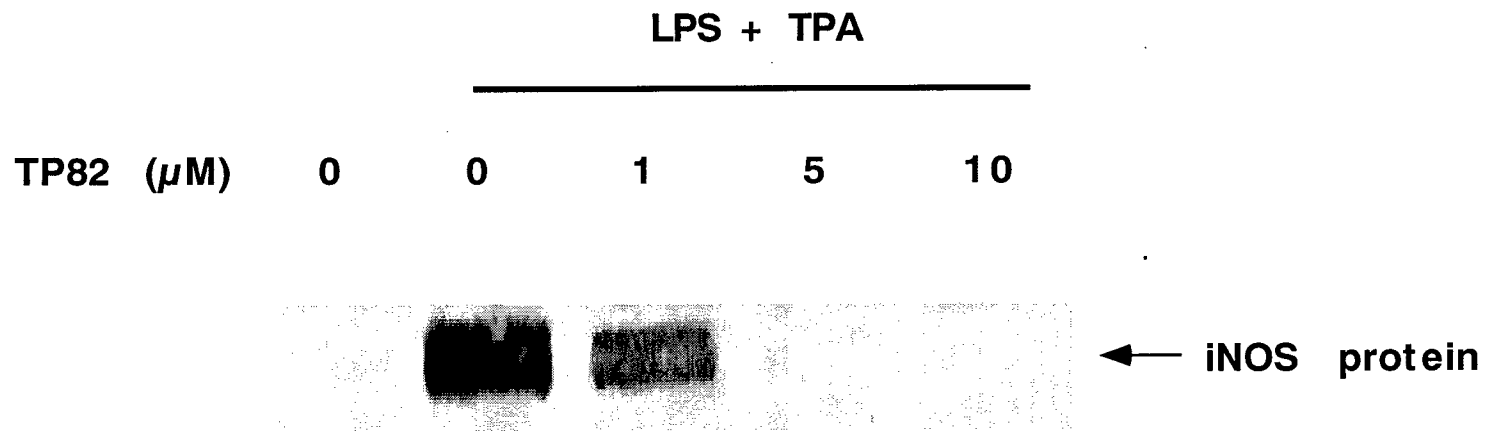
NRP152 and NRP-154 cells were treated with LPS (10 ng/ml), TPA (10 ng/ml) or with LPS plus TPA in the presence or absence of different concentrations of compounds for 12 h. Cell lysates were obtained and used for western analysis for iNOS or COX-2 expression.

Repression of iNOS protein by TP151 (CDDO) in NRP-152



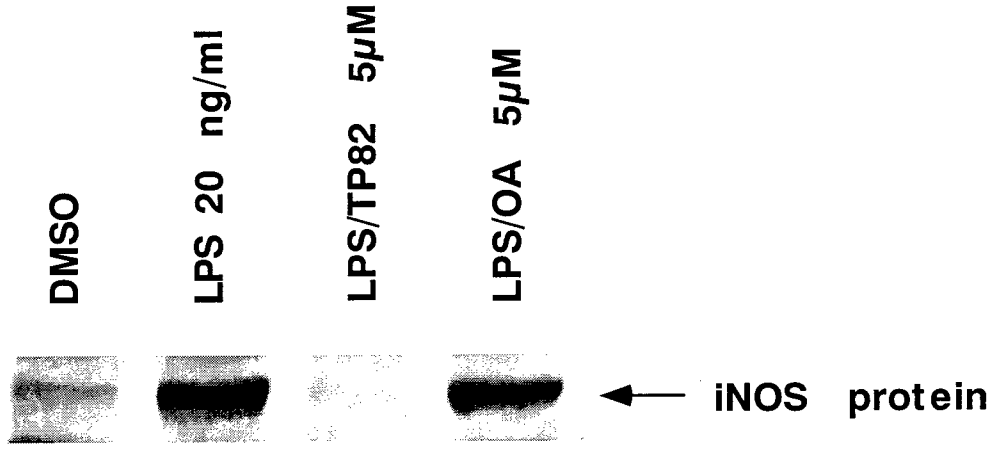
Exponentially growing NRP152 cells were treated with LPS with or without TP151 at indicated concentrations for 12 h. Cell lysates were harvested and subjected to western analysis.

Repression of iNOS by TP82 in NRP152



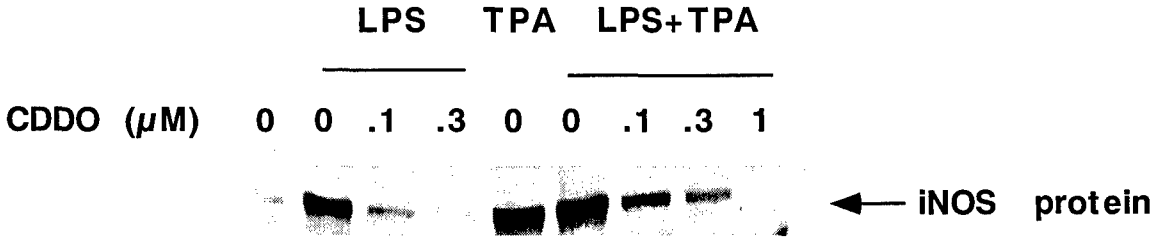
NRP152 cells were treated with LPS (10 ng/ml) and TPA (10 ng/ml) in the presence or absence of different concentrations of compounds for 12 h. Cell lysates were obtained and used for western analysis for iNOS or COX-2 expression.

Comparison of TP82 and OA in regulation of iNOS protein in NRP-152



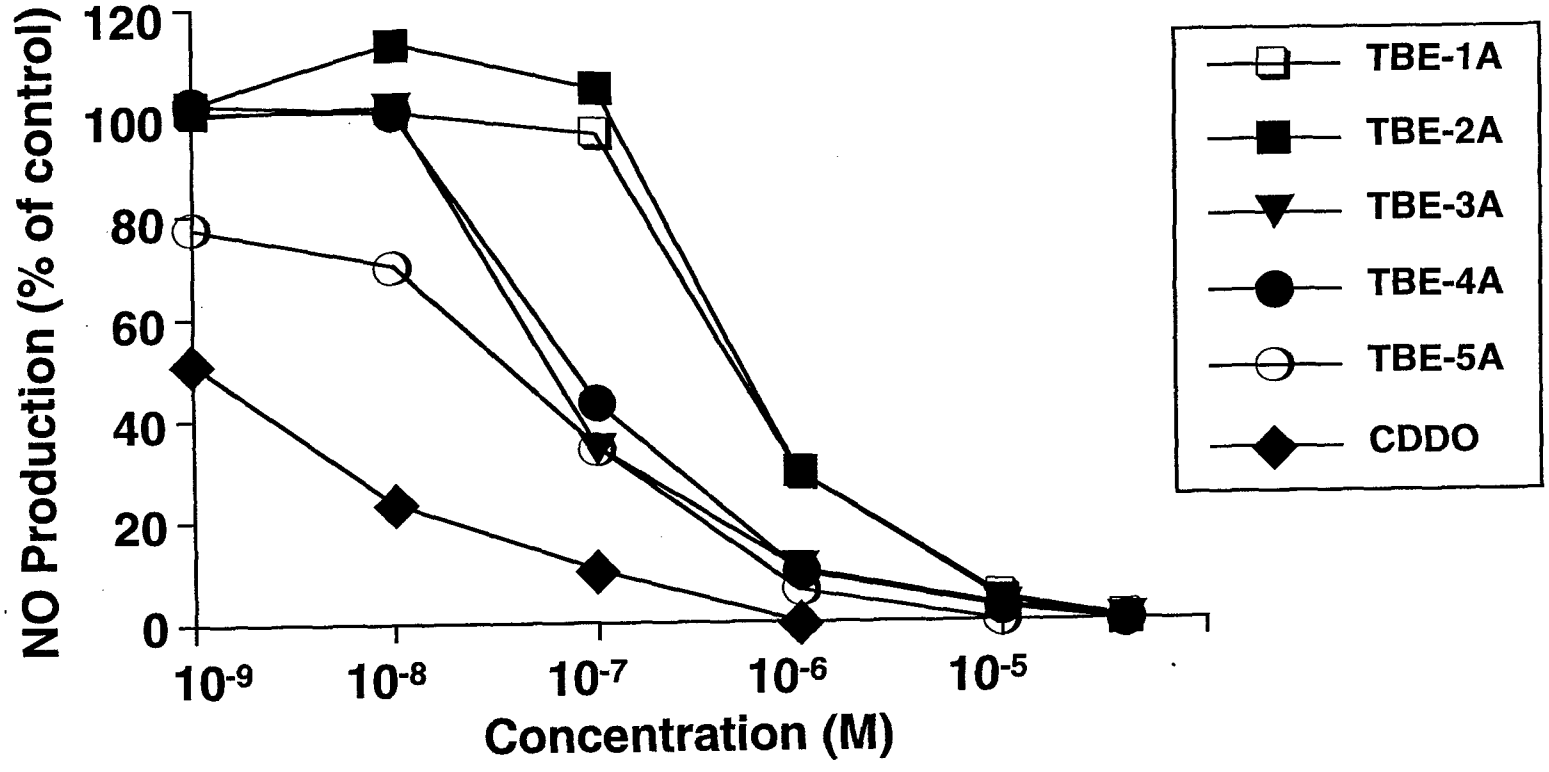
Exponentially growing NRP152 cells were treated with LPS, with LPS and TP82 combination or with LPS and OA combination at indicated concentrations for 12 h. Cell lysates were harvested and subjected to Western analysis.

Repression of iNOS protein by TP151 (CDDO) in NRP-152



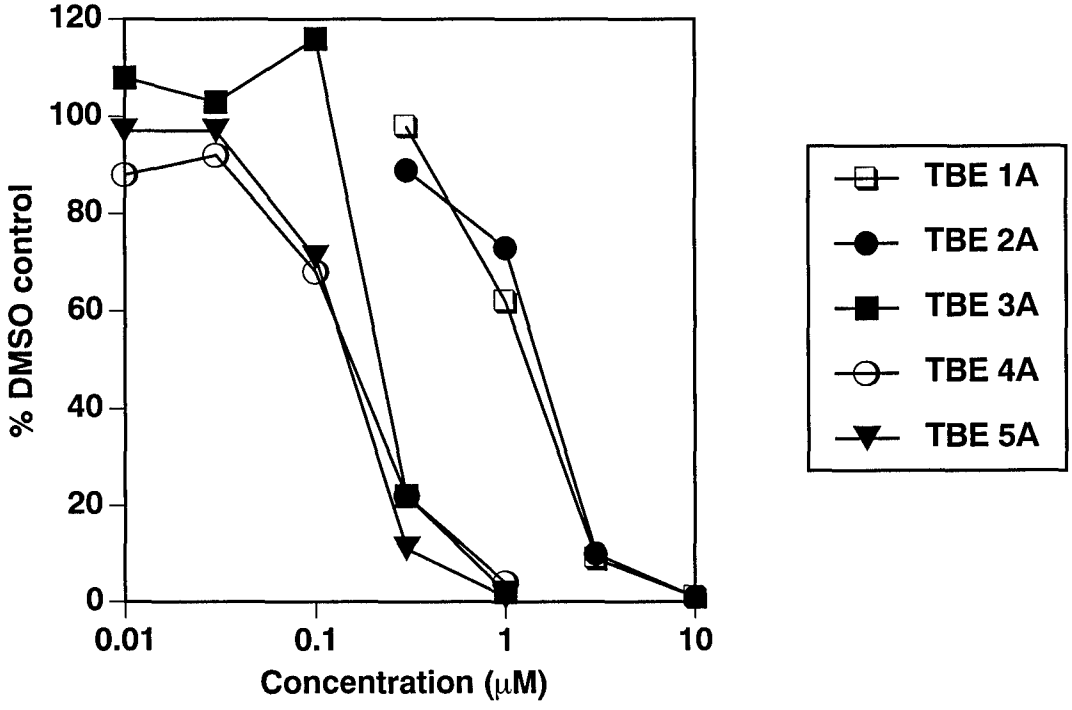
NRP152 cells were treated with LPS (10 ng/ml), TPA (10 ng/ml) or with LPS plus TPA in the presence or absence of different concentrations of TP151 (CDDO) for 12 h. Cell lysates were obtained and used for western analysis for iNOS expression.

TBEs inhibit nitric oxide production in primary mouse macrophages induced by interferon-γ



10/1/99 TBEs inhibit nitric oxide (NO) production in primary macrophages. IFN-γ (40 ng/ml) was used to induce nitric oxide production in mouse macrophages. The cells were incubated for 48 hrs with the inducer and compounds, then nitric oxide in the supernatant was measured by Griess Reaction.

Tricyclic Bis-Enone Compounds on NRP-152 Cells



11-8-99 Tricyclic bis-enone (TBE) compounds inhibit growth of NRP-152 rat prostate cells. Cells were incubated with TBEs for 72 hours in DMEM/F12 media containing 1% charcoal-stripped serum, insulin, dexamethasone, and HEPES. Growth inhibition was measured by 3H-thymidine incorporation.

KEY RESEARCH ACCOMPLISHMENTS

- First synthesis of new tricyclic bis-enones structurally related to pentacyclic triterpenoids
- Demonstration of potent activity of new triterpenoid, CDDO, for suppression of growth of prostate epithelial cells
- Demonstration of potent activity of new triterpenoid, CDDO, for suppression of de novo induction of iNOS mRNA and iNOS protein in prostate epithelial cells
- Demonstration of ability of new tricyclic bis-enones to block induction of iNOS in primary mouse macrophages
- Demonstration of ability of new tricyclic bis-enones to inhibit growth of prostate epithelial cells

REPORTABLE OUTCOMES

Manuscript in press "Novel Synthetic Oleanane Triterpenoids: A series of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages" by Tadashi Honda, BarbieAnn V. Rounds, Lothar Bore, Frank G. Favaloro, Jr., Gordon W. Gribble, Nanjoo Suh, Yongping Wang, and Michael B. Sporn, Bioorganic & Medicinal Chemistry Letters, to be published in year 2000

Bioorganic and Medicinal Chemistry Letters (In Press)

NOVEL SYNTHETIC OLEANANE TRITERPENOIDS: A SERIES OF HIGHLY ACTIVE INHIBITORS OF NITRIC OXIDE PRODUCTION IN MOUSE MACROPHAGES

Tadashi Honda,[a] BarbieAnn V. Rounds,[a] Lothar Bore,[a] Frank G. Favaloro, Jr.,[a] Gordon W. Gribble,*[, a] Nanjoo Suh,[b] Yongping Wang,[b] and Michael B. Sporn*[, b]

[a]*Department of Chemistry, Dartmouth College, Hanover, NH 03755, U.S.A. and*
[b]*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, U.S.A.*

Abstract: Novel oleanane triterpenoids with modified rings A and C were designed and synthesized. Among them, methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate showed similar high inhibitory activity (IC_{50} = 0.8 nM) to 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), which we have synthesized previously, against production of nitric oxide induced by interferon-γ in mouse macrophages.

Introduction

In a previous communication[1] we reported that 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) (**1**) has high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50} = 0.1 nM level). We also showed that CDDO is a potent, multifunctional agent.[2] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. CDDO inhibits proliferation of many human tumor cell lines. CDDO blocks *de novo* synthesis of inducible nitric oxide synthase (*i*-NOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. CDDO will protect rat brain hippocampal neurons from cell death induced by β-amyloid. The above activities have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture.

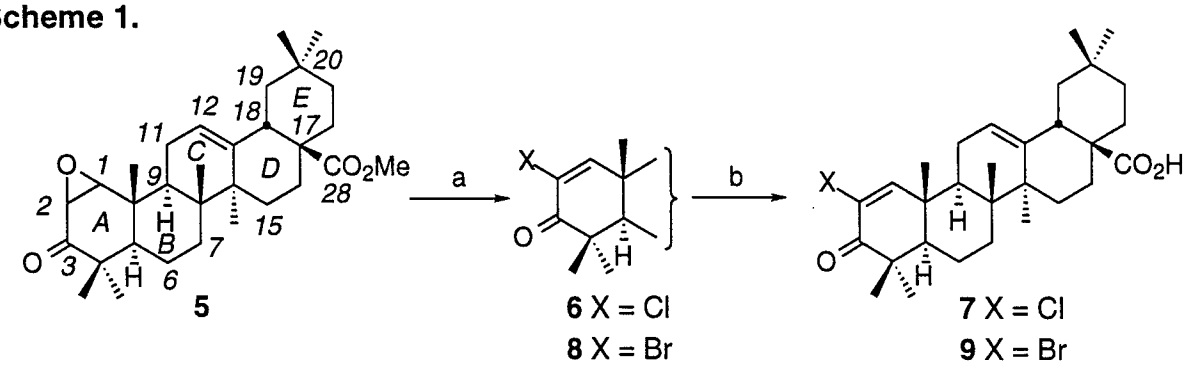
In the communication,[1] we also reported that the combination of a 1-en-3-one functionality with a nitrile group at C-2 in ring A and a 9-en-12-one functionality in ring C enhances activity very strongly in comparison with the enhancement by each functionality alone. We therefore designed and synthesized a series of novel oleanane triterpenoids to survey what combination of ring A with ring C provides highly active compounds. We have found that methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate (**2**) has similar high inhibitory activity to CDDO and methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO methyl ester) (**3**).[1,3] The new compound **2** is expected to be an alternative agent to CDDO. In this communication, the synthesis, inhibitory activity, and structure–activity relationships (SAR) are reported for these analogs.

Chemistry

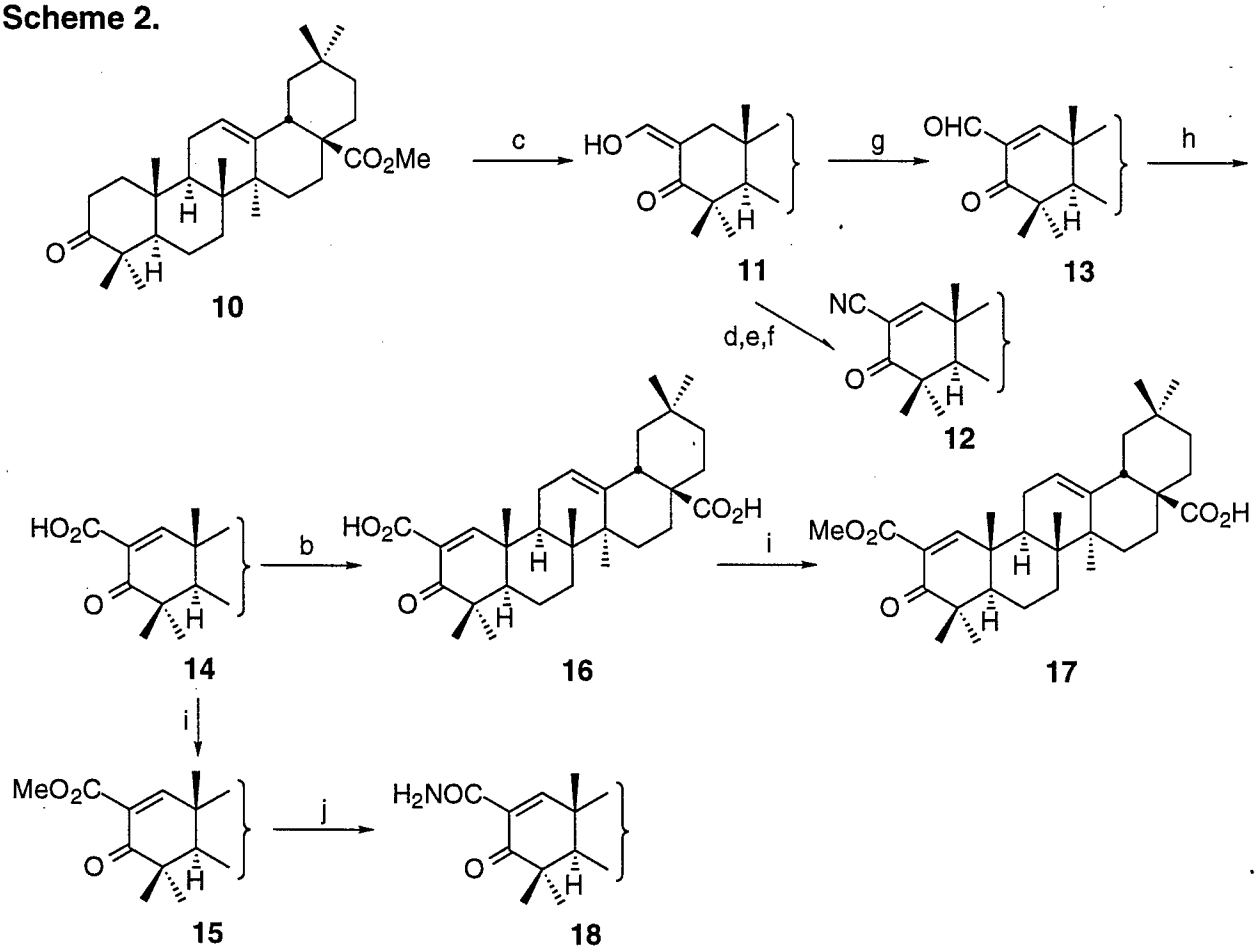
Modification of Ring A (Schemes 1 and 2)

Initially, we designed and synthesized new olean-12-ene derivatives with a 1-en-3-one functionality having a substituent at C-2 in ring A, **6–9** and **12–18**, to discover which substituents enhance activity in comparison with the lead compound **4**, which was reported previously.[4] Chloride **6** was synthesized in 81% yield from

Scheme 1.

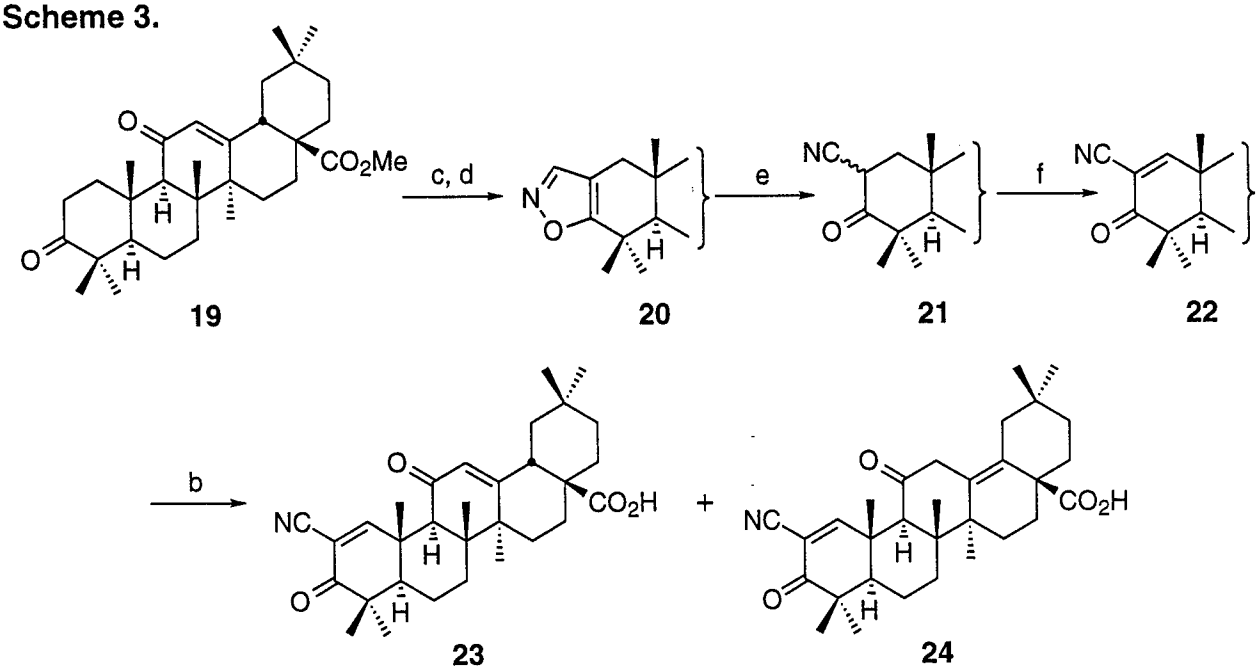


Scheme 2.

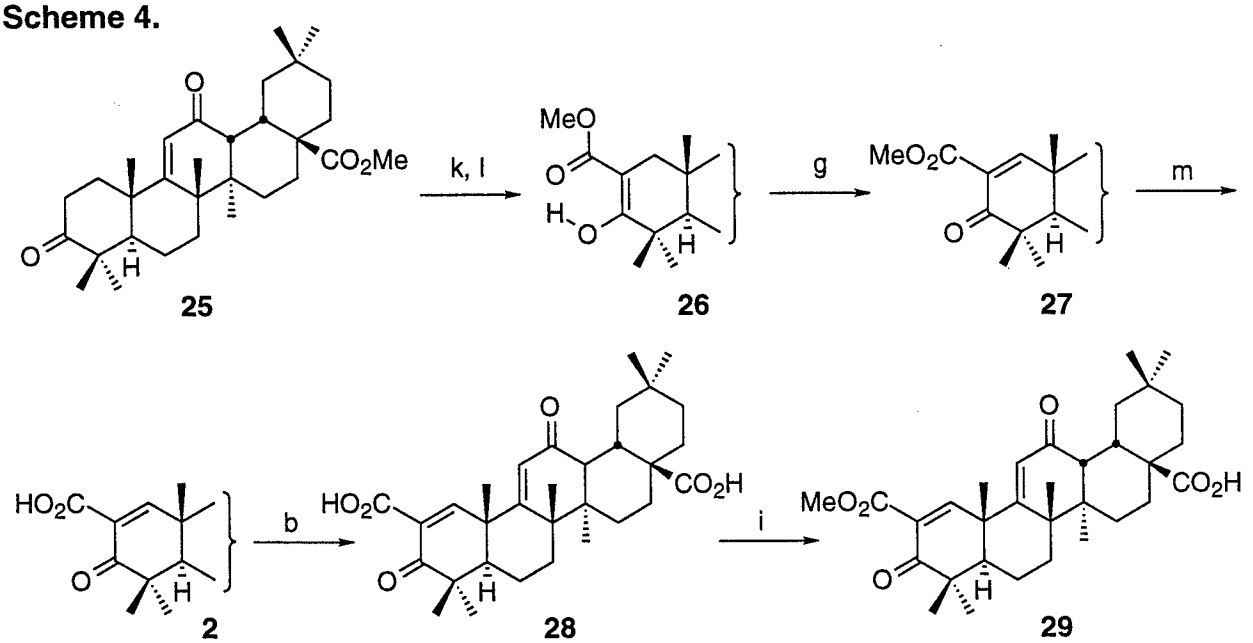


epoxide **5**[4] with hydrogen chloride in acetic acid and $CHCl_3$.[5] Halogenolysis of **6** with LiI in DMF[6] gave chloride **7** in 77% yield. Similarly, bromides **8** and **9** were prepared from **5** and **8** (yield, 96% and 76%), respectively. Compound **11**[7] was prepared in 95% yield by formylation of C-3 ketone **10**[4] with ethyl formate in the presence of sodium methoxide in benzene.[8] Nitrile **12** was synthesized in three steps (yield, 30%) from **11** according to the same synthetic route as for **30**, which was prepared previously.[1] Enal **13** was prepared from **11** by phenylselenenyl chloride-pyridine in CH_2Cl_2 and sequential addition of 30% H_2O_2[9] (yield, 71%; 79% based on recovered **11**). Jones oxidation of **13** gave acid **14** in 30% yield. Methylation of **14** with MeOH under acidic conditions gave ester **15** in 80% yield. Halogenolysis of **14** gave dicarboxylic acid **16** in 58% yield. Methylation of **16** with MeOH under acidic conditions gave ester **17** selectively in 70% yield because the carboxylic acid at C-17 of **16** is very sterically hindered. Amide **18** was prepared selectively in 72% yield from **15** with saturated ammonia-MeOH. Compounds **12** and **14–17** were found to be more active than the lead compound **4** (see Table 1).

Scheme 3.



Scheme 4.



a: HX/AcOH/$CHCl_3$, b: LiI/DMF, c: HCO_2Et/NaOMe/PhH, d: NH_2OH·HCl/aq EtOH, e: NaOMe/Et_2O/MeOH, f: PhSeCl/AcOEt; 30%H_2O_2/THF, g: PhSeCl/pyr./CH_2Cl_2; 30%H_2O_2/CH_2Cl_2, h: Jones, i: H_2SO_4/MeOH, j: NH_3/MeOH, k: Stiles' reagent/DMF, l: CH_2N_2/Et_2O/THF, m: KOH/aq MeOH

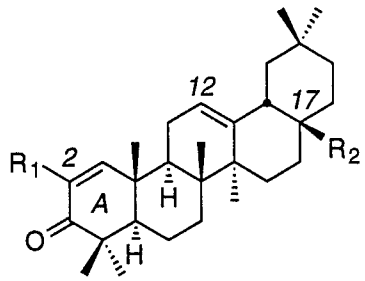
Modification of Ring C

We already reported the synthesis and inhibitory activity of 3-oxoolean-1-ene derivatives with various structures of ring C, and among them enones **31–33** are more active than the lead compound **4** (see Table 2).[4]

Combination of Modified Ring A with Ring C (Schemes 3 and 4)

On the basis of the above results, new oleanane derivatives with modified rings A and C, **2**, **22–24**, and **27–29**, were designed and synthesized. Isoxazole **20** was prepared from C-3 ketone **19**[4] by formylation (yield, 98%), followed by condensation with hydroxylamine (yield, 74%).[10] Cleavage of the isoxazole moiety of **20** with sodium methoxide gave nitrile **21** in 92% yield.[10] Nitrile **22** was prepared from **21** by phenylselenenyl

Table 1. IC_{50} (μM)[a] Values of Olean-12-ene Derivatives with Modified Ring A



compd	R_1 at C-2	R_2 at C-17	Taft's σ^* value of R_1	activity IC_{50} (μM)
34 [4]	OH	CO_2H	1.34	27
18	$CONH_2$	CO_2Me	1.68	14
35 [4]	OMe	CO_2H	1.81	30
15	CO_2Me	CO_2Me	2	0.9
17	CO_2Me	CO_2H		2.2
14	CO_2H	CO_2Me	2.08	0.8
16	CO_2H	CO_2H		0.07
13	CHO	CO_2Me	2.15	toxic[b]
36 [1]	CHO	CO_2H		toxic[b]
8	Br	CO_2Me	2.84	> 40
9	Br	CO_2H		7.3
6	Cl	CO_2Me	2.96	> 40
7	Cl	CO_2H		> 40
12	CN	CO_2Me	3.3	0.7
30 [1]	CN	CO_2H		0.6
4 [4]	H	CO_2H	-	5.6
oleanolic acid			-	> 40
hydrocortisone			-	0.01

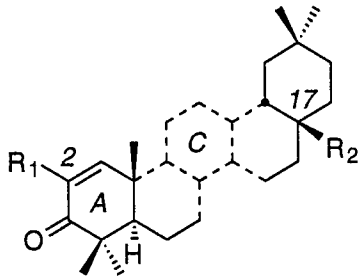
chloride in ethyl acetate and sequential addition of 30% H_2O_2[11] (yield, 33%; 57% based on recovered **21**). Halogenolysis of **22** gave acids **23** and **24** in 37% and 16% yield, respectively. Compounds **2** and **27–29** could not be synthesized according to the similar synthetic route as for **14–17** because Jones oxidation of the precursor of **2** (aldehyde at C-2) gives an unknown compound instead of **2**. They were synthesized according to the alternative route illustrated in Scheme 4. Ester **26** was prepared in 78% yield from C-3 ketone **25**[4] by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,[12] followed by methylation with diazomethane. Enone **27** was prepared from **26** according to the same method as for **13** (yield, 71%; 88% based on recovered **26**). Hydrolysis of **27** with potassium hydroxide in aqueous MeOH gave acid **2** selectively in 78% yield again because of the steric hindrance of the methoxycarbonyl group at C-17 of **27**. Halogenolysis of **2** gave dicarboxylic acid **28** and monocarboxylic acid **31** in 47% and 24% yield, respectively. Methylation of **28** with MeOH under acidic conditions gave ester **29** selectively in 82% yield.

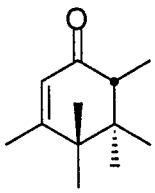
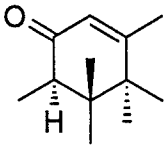
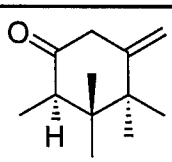
Biological Results and Discussion

Inhibitory Activity of Olean-12-ene Derivatives with Modified Ring A

The inhibitory activities [IC_{50} (μM) value] of olean-12-ene derivatives with a 1-en-3-one functionality with a substituent at C-2 in ring A,[13] oleanolic acid, and hydrocortisone (a positive control) on production of NO induced by IFN-γ in mouse macrophages[14] are shown in Table 1. These derivatives are arranged according to

Table 2. IC_{50} (μM)[a] Values of Oleanane Derivatives with Modified Rings A and C



compd	structure of ring C	R_1 at C-2	R_2 at C-17	activity IC_{50} (μM)
3 [1]		CN	CO_2Me	0.0001
1 [1]		CN	CO_2H	0.0002
27		CO_2Me	CO_2Me	toxic[b]
29		CO_2Me	CO_2H	0.1
2		CO_2H	CO_2Me	0.0008
28		CO_2H	CO_2H	0.2
31 [4]		H	CO_2H	0.2
22		CN	CO_2Me	0.02
23		CN	CO_2H	0.04
32 [4]		H	CO_2H	1.4
24		CN	CO_2H	0.07
33 [4]		H	CO_2H	2.6
dexamethasone				0.0001

[a]IC_{50} (μM) values of compounds **1–3**, **16**, **22–24**, hydrocortisone and dexamethasone were determined in the range of 0.1 pM–1 μM (tenfold dilutions). The other compounds were assayed in the range of 0.01–40 μM (fourfold dilutions). Values are an average of two separate experiments.
[b]Compounds **13**, **27** and **36** were toxic to cells above 1 μM and were not active below 1 μM.

the strength of Taft's σ* values[15] of substituents at C-2. These results provide the following interesting SAR:

(1) The relationship between Taft's σ* value and activity is not observed.

(2) Methoxycarbonyl, carboxyl, and nitrile groups at C-2 enhance activity. Compounds **12**, **14–16**, and **30** are about 10–100 times more active than the lead compound **4**.

(3) Hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decrease activity.

(4) Formyl group does not show activity, but only toxicity.

(5) Methoxycarbonyl and carboxyl groups at C-17 show similar activity.

Inhibitory Activity of Oleanane Derivatives with Modified Rings A and C

The inhibitory activities [IC_{50} (μM) value] of oleanane derivatives with modified rings A and C,[13] and dexamethasone (a positive control) on production of NO induced by IFN-γ in mouse macrophages are shown in Table 2. These results provide the following interesting SAR:

(1) A 9-en-12-one functionality is the strongest enhancer of activity among structures of ring C. Compound **31** is about 10 times more active than **4**.

(2) 12-En-11-one and 13-en-11-one functionalities also enhance activity. Compounds **32** and **33** are about 2–4 times more active than **4**.

(3) The combination of a 9-en-12-one functionality with nitrile and carboxyl groups at C-2 provides extremely highly active compounds. Compounds **2**, **3**, and CDDO (**1**) are about 10,000 times more active than **4**.

(4) The combination of 12-en-11-one and 13-en-11-one functionalities with a nitrile group at C-2 also provides highly active compounds. Compounds **22–24** are about 100 times more active than **4**.

(5) Although compounds **27–29** were also expected to show similar high activity to CDDO from the perspective of SAR, they did not show high activity.

Currently, further evaluation in vivo for both antiinflammation and chemoprevention of CDDO, **2**, and **3** are in progress. Studies on the mode of action of these compounds also are in progress.

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3. CDDO methyl ester (**3**) was found to show the same high activity as CDDO after our previous communication[1] was published.
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7. ^{1}H and ^{13}C NMR of compound **11** in $CDCl_3$ showed that it is the single isomer as depicted in Scheme 2.
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13. All new compounds, **2**, **6–9**, **12–18**, **22–24**, and **27–29** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses.
14. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days earlier with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 20 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 16.
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